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(54) Title: PROCESS FOR THE PRODUCTION OF A PROTEIN-VITAMIN FOOD SUPPLEMENT FROM AUTOTROPHIC MICRO-ORGANISMS, IN PARTICULAR FROM ALGA *SPIRULINA*

(57) Abstract

Alga *Spirulina* is subjected successively to hydrothermal and proteolytic enzymatic attack, and to alcoholic extraction, in order to yield a protein-vitamin food supplement which can be used in the formulation of protein or high-protein vegetarian foodstuffs.

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PROCESS FOR THE PRODUCTION OF A PROTEIN-VITAMIN FOOD SUPPLEMENT FROM AUTOTROPHIC MICRO-ORGANISMS IN PARTICULAR FROM ALGA SPIRULINA

Background Art

A food supplement of this invention has an advantageous and fundamental application in the formulation of protein or high-protein food products in which the protein content is not derived from animal meat. These food products, numerous and varied according to local requirements and/or quotas of consumption, represent a new line in vegetarian dietetics.

The following amino-acids are a necessary part of the human diet (essential amino-acids): isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, valine and lysine.

In the diet of man and of other animals, proteins are in practice the sole source of essential amino-acids, and this is the reason for the well-known and general interest in protein production for human nutrition.

It is likewise known that the autotrophic micro-organisms and, among these, the algae, are potentially the main sources of protein of non-conventional origin.

With regard to the use of algae in human foodstuffs, only rather unsatisfactory results have been obtained from attempts to add them directly, where appropriate in mixtures with other food products, for example flour. Even with limited daily doses, there have been drawbacks, for example oedema of the face and hands, petechial haemorrhages, cyanosis of the nails, general intestinal problems, and these have rapidly discouraged their use. Some of these drawbacks had a less noticeable effect when the algae were subjected to prior physico-chemical treatments to remove or reduce their colour.

In addition to the abovementioned drawbacks of direct administration, there is also the problem of the taste which cannot easily be neglected. For the majority of people, its intensity and unpleasantness has been a basic reason for rejecting the use of these algae in foodstuffs. This problem could be largely overcome, according to a technical suggestion which appeared in Chemical Abstract 91,90329, by a treatment which

removes the bitter taste: this consists mainly in boiling or cooking the fresh algae for a period of 10-15 minutes.

However, the drawback which limits the direct use of the algae in human foodstuffs is the high concentration of nucleic acids, which are known to cause dangerous levels of uric acid in the blood. It is further known that, of all the algae used for alimentary purposes, alga Spirulina is the most important, since it contains all of the essential amino-acids mentioned above and since it is the one which has been most widely tested and utilised.

A great deal has been reported, in fact, on its use by the people who live on the banks of Lake Chad in Africa, where alga Spirulina has been an ingredient of their daily diet for several hundred years. This alga was also used by the Aztecs in ancient Mexico. However, this information remains anecdotal in character, since from a strictly technical point of view, there is little information available on the quantities of alga Spirulina consumed by these people. The only certain fact is that this alga, which is especially rich in the basic components of human nutrition, can be administered up to a maximum of 26 g per day as a dry substance, without there being any apparent side-effects. Although alga Spirulina has been produced both in the laboratory as well as in small pilot plants in France and Mexico, published results indicate that its acceptability to man is very limited.

Disclosure of the Invention

Precisely because of the high and valuable protein content of alga Spirulina, the problem underlying the present invention is to provide a process for obtaining a protein-vitamin food supplement which can be used in the formulation of protein or high-protein food products, or actually added as such, overcoming the abovementioned drawbacks of the prior art.

According to the present invention, this problem is resolved by a process which consists of the successive stages of:

- boiling and cooking a predetermined quantity of alga Spirulina for a period of between 10 and 15 minutes,
- proteolytic enzymatic attack of the cooked Spirulina, after cooling it to about 20-45°C,

- subsequent centrifugation so as to obtain an aqueous extract containing most of the water-soluble molecules present in the original alga Spirulina, and a moist residue,
- alcoholic extraction of the said residue, previously heated to about 80°C, with ethyl alcohol at 95°C,
- centrifugation so as to obtain an alcoholic extract and a residue forming a protein food supplement containing up to 83% protein, up to 11% carbohydrate and up to 3% fat.

Alga Spirulina subjected to the process of this invention can be fresh or in powder form.

In the first case, having a water content of up to 85%, the alga Spirulina is cooked as such. In the second case, it will be added with an appropriate quantity of water.

The 10-15 minute cooking (boiling) stage is sufficient to change the taste of the alga Spirulina, owing to substantial steam distillation of some of the substances responsible for the bitter after-taste, which is consequently completely absent from the protein food supplement obtained using the process of this invention.

During the same abovementioned cooking stage , coagulation of the larger proteins contained in the alga Spirulina takes place; this coagulation facilitates the predigestive task of proteolytic enzymatic attack. This hydrolytic attack has several purposes: it liberates extractable elements, it reduces the molecular complexity of certain proteins with the consequent formation of free amino-acids, and it confers an acceptable, if not pleasant, taste to the protein food supplement obtained. The two-fold attack, hydrothermal and enzymatic, also ensures the breakdown of the cell wall, which, fortunately, is mucopolysaccharide and not cellulose in character.

The aqueous extract from the first stage of centrifugation, carried out after cooking the alga Spirulina, contains all the water-soluble molecules and especially the mineral salts which occur in notoriously excessive amounts in the original alga. In accordance with this invention, this extract is fractionated in order to recover the vitamins, water-soluble proteins and polysaccharides, which are eventually recycled into the protein food supplement so as to restore its original biological value.

Still in accordance with this invention, the alcoholic extract containing fats, vitamins, styrols, cholesterol, oligopeptides, beta-carotene, chlorophyll, blue pigment and other small molecules present in the original alga Spirulina is also fractionated in order to recover valuable components as well as the ethyl alcohol itself which is recycled to the alcoholic extraction stage.

Advantageously and in accordance with a further characteristic feature of this invention, the protein food supplement obtained by the process outlined above is cooked with flours and/or tubers - such as wholemeal flour, whole millet, whole sorghum, whole manioca flour and yam and/or potato flour for example - either in the fresh state, or ground, and after being partially or totally dried.

Further characteristic features and advantages of the process of this invention will become clearer from the following description of an example of its implementation, given only by way of example, and of several examples of formulations of European-type vegetarian food products containing the protein supplement obtained by the abovementioned process.

Modes for Carrying Out the Invention

1500gm of alga Spirulina in dry powder form with a residual moisture content of 6% were loaded into a 10 litre batch reactor together with 10 litres of water.

The contents were brought to boiling, and this was maintained for 15 minutes in order to cook the algae adequately. This operation caused, on the one hand, coagulation of the larger proteins and, on the other hand, substantial steam distillation of the bitter-tasting substances originally present in the alga Spirulina.

After cooling the contents to 40°C, a mixture of proteolytic enzymes was added to the reaction vessel. In particular, the preferred enzymatic mixture included:

amylase	-	400.5 units
lipase	-	45 units
trypsin	-	48,000 units
chymotrypsin	-	36,000 units
cellulose	-	60 mg

The enzymatic attack stage was allowed to last for 4 hours 15 minutes, and a suspension was obtained which was filtered under vacuum on a Buchner type filter.

The aqueous extract was sent for fractionation, from which the mineral salts, vitamins and water-soluble proteins were obtained.

The filter-cake obtained from the abovementioned filtration was pressed and returned to the 10-litre batch reactor, together with 3 litres of good quality ethyl alcohol at 95°C. The total contents underwent reflux distillation for 1 hour 10 minutes. Subsequently, the contents were filtered whilst hot on a Buchner type filter under vacuum.

The filter-cake obtained from this latter filtration was pressed and washed once with 1000 cc of distilled water after removing the alcoholic eluate.

On analysis, the water-washed protein filter-cake had a residual humidity of 50%; analysis of the dried material indicated the following composition in terms of percentage weight:

ash	2.45
protein	83.2
carbohydrate	11.0
fat	2.7
fibre	0.65

This protein filter-cake which constitutes the protein food supplement of this invention, was subdivided into five portions each of 800 g wet weight (approx. 260 g dry material). The portions were kept in a refrigerator from which samples were taken to be formulated into a protein foodstuff. Some examples of these formulations are given below.

EXAMPLE 1

A portion of protein supplement was loaded into a 10 litre batch reactor with 2 kg of wholemeal flour and 3 litres of water. The total contents were cooked for 20 minutes until the flour was gelatinised. After cooling, the mixture obtained was loaded into a double-Z type food-mixer with an effective volume of 5 litres. 8 eggs and 1 kilogram of flour were loaded into the same machine.

After obtaining a homogeneous mixture, the latter was rolled into a thin sheet and cut in the shape of tagliatelle.

The tagliatelle, cooked and seasoned in the normal way, left no after-taste in the mouth when sampled.

EXAMPLE 2

A portion of the abovementioned protein food supplement was loaded into a double-Z type food-mixer together with bread crumbs, parmesan cheese, meat extract, spices, natural meat flavourings and four eggs.

After obtaining a homogeneous mixture, the latter was subdivided to make rissoles with a diameter of about 5 cm. The rissoles were fried in plentiful oil, and left no after-taste in the mouth on sampling.

EXAMPLE 3

A portion of the protein food supplement obtained using the procedure of this invention was loaded into a double-Z type food-mixer together with sugar, eight eggs, milk and vanilla sugar.

After obtaining a homogeneous and slightly fluid mixture, the latter was poured into a cake tin, and the cake obtained, when sampled, had no after-taste reminiscent of the original alga Spirulina.

EXAMPLE 4

A further portion of the protein food supplement of this invention was cooked with flour as in Example 1 and subsequently dried under vacuum in the 10 litre batch reactor. The dry material was ground in order to obtain an instantaneous semolina with a pleasant flavour. No after-taste remained in the mouth when this food product was sampled.

EXAMPLE 5

A portion of the protein supplement of this invention was placed in a double-Z type food-mixer together with sugar, dextrin and a little milk. After obtaining a homogeneous mixture, the latter was loaded into a batch reactor under vacuum in order to reduce the moisture content to about 25%; this allowed subsequent rolling

and pressing out of biscuits by hand; these were dried in an oven.

Crisp biscuits were obtained with the specific taste of so-called breakfast biscuits. No after-taste was left in the mouth reminiscent of the original alga Spirulina.

CLAIMS

1. Process for the production of a protein-vitamin food supplement from alga Spirulina, characterised in that it consists of the successive stages of:

- boiling and cooking of a predetermined quantity of alga Spirulina for a period of between 10 and 15 minutes,
- proteolytic enzymatic attack of the cooked Spirulina, after cooling it to a temperature of about 40°C,
- centrifugation so as to obtain an aqueous extract containing the water-soluble molecules present in the original alga Spirulina and a moist residue,
- alcoholic extraction of the abovementioned moist residue, previously heated to about 80°C, with ethyl alcohol at 95°C,
- centrifugation so as to obtain an alcoholic extract and a residue forming a protein food supplement containing up to 83% protein, up to 11% carbohydrate and up to 3% fat.

2. Process according to Claim 1, characterised in that the aqueous extract obtained by centrifugation following the proteolytic enzymatic attack stage is fractionated in order to recover the water-soluble molecules, in particular the mineral salts, the vitamins, the water-soluble proteins and the water-soluble polysaccharides present in the original alga Spirulina.

3. Process according to Claim 2, characterised in that at least a portion of the said water-soluble molecules are recycled into the protein food supplement.

4. Process according to Claim 1, characterised in that the alcoholic extract is fractionated in order to recover the ethyl alcohol and re-use it in the alcoholic extraction stage.

5. Process according to Claim 1, characterised in that the said protein food supplement is cooked with cereal and/or tuber flours so as to obtain the respective mixtures.

6. Process according to Claim 5, characterised in that the said mixtures are moulded in the conventional manner.

7. A protein food supplement characterised in that it is obtained using the process of Claim 1.

8. A protein food supplement characterised in that it is obtained using the process of Claim 3.

9. A protein-vitamin food of vegetable origin characterised in that it consists of a mixture made from a protein food supplement of Claim 7 cooked with a cereal and/or tuber flour.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 86/00439

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [5]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 23 J 1/00; A 23 L 1/305

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 23 J; A 23 L

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	Journal of the Agricultural and Food Chemistry, vol. 29, no. 3, May/ June 1981 (Washington, Columbia, US) M. Anusuya et al.: "Studies on the hoteins of mass-cultivated, blue-green alga (spirulina platensis)", pages 522-525, see page 522, column 2; page 523, column 2 and page 525, column 1	1,7
A	FR, E, 95866 (INSTITUT FRANCAIS DU PETROLE DES CARBURANTS & LUBRIFIANTS) 12 November 1971 see claims 1-8; page 3, lines 3-7; examples 3,4	9
A	EP, A, 0079023 (HOECHST) 18 May 1983 see claims 1,4,6,9	1,7

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
20th November 1986	14 JAN. 1987
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: J. VAN MOL

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/EP 86/00439 (SA 14248)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 03/12/86

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR-E- 95866	12/11/71	None	
EP-A- 0079023	18/05/83	DE-A- 3143947	11/05/83
		JP-A- 58081740	17/05/83
		AU-A- 9014482	12/05/83
		AU-B- 554815	04/09/86